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SecYEG

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Chapter 5

Summary and perspective

Introduction

The bacterial world consists of a wide variety of single celled organisms constantly adapting to and interacting with the surrounding environment. The intricate machinery allowing life inside these organisms is protected from the outside world by one or more surface layers. An essential barrier that separates the inside from the outside of the cell is the inner membrane, which is composed of a lipid bilayer packed with membrane proteins. This barrier shields the cytosol from harmful compounds, which can disturb the delicate metabolic balance in the cell. Conversely, this barrier also prevents bacteria from releasing compounds among which proteins. For this purpose, specialized transporter complexes are present in the inner membrane that mediate the selective secretion of proteins that function outside of the cell. This process has been extensively researched in the model organism *Escherichia coli*. The majority of secretory proteins in *E. coli* are translocated across the inner membrane by the Sec machinery. Secretory proteins carry a signal sequence that guides them to the SecA motor protein which associates with the SecYEG translocation channel and subsequently drives the translocation of these secretory proteins across the membrane at the expense of the energy source ATP. Once the translocating protein emerges at the other side of the channel it interacts with the accessory complex SecDF, which pulls the protein out the channel at the expense of the proton motive force. **Chapter 1** summarized our current insight and views on the mechanisms of bacterial protein translocation and integrates this knowledge in a proposed mechanism of functioning.

Role of the SecY plug domain

The membrane channel SecYEG accommodates protein translocation as well as insertion of proteins in the lipid bilayer. The crystal structure of the *Methanocaldococcus jannaschii* SecYEβ [8] shows that the SecY subunit forms the actual channel. SecY is enwrapped in a V-shaped manner by the small integral membrane protein SecE (Figure 1). The Secβ subunit, which is homologous to the *E. coli* SecG, is located more peripherally in the structure. SecY is composed of ten

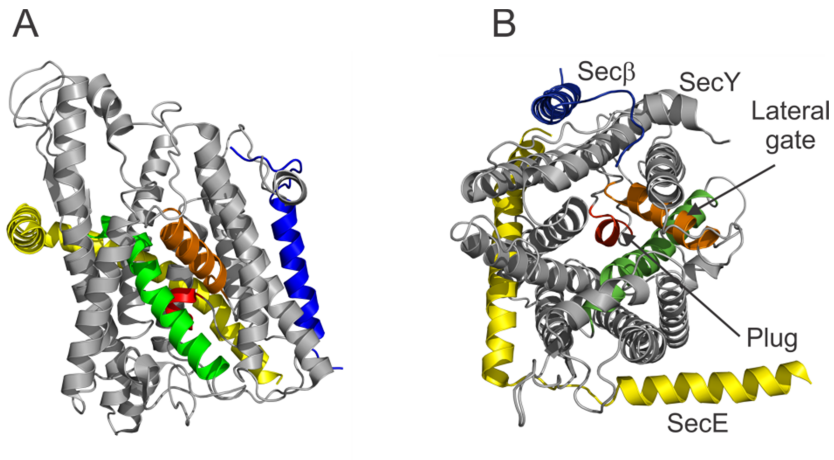

Figure 1: Crystal structure of SecYEβ from *M. jannaschii* (PDB code: 1RH5) shown from a (**A**) side- and (**B**) top-view (from the cytoplasm). In color are indicated SecY (gray) with the plug domain (red), the lateral gate helices TMS2b (orange) and TMS7 (green), SecE (yellow) and Secβ (blue).

transmembrane segments (TMSs) which are arranged in a clamshell fashion where TMS 1-5 and TMS 6-10 form two halves which are connected by a hinge region. In a cross-section, the SecY channel has an hourglass shape with a constriction ring in the middle, which is lined by hydrophobic residues. At the periplasmic side, and in close association with the constriction ring, a small re-entrance loop forms a short α-helix termed the plug domain. When viewed from the cytoplasm the plug domain clearly obstructs a vectorial pathway through the SecY channel and has to relocate to allow secretory protein to pass the channel. Early crosslinking studies proposed that during protein translocation the plug domain moves away from the constriction ring to the tip of SecE by more than 27 Å [56,57]. This would be a very large structural rearrangement and the question was addressed: what is the minimal conformational change of the plug domain needed to support protein translocation. **Chapter 2** describes an extensive crosslinking study where we immobilize the plug domain inside the channel and analyze its effect on protein translocation. Using a range of crosslinkers we were able to crosslink the plug domain to TMS10 of the SecY channel at different distances of 2, 8 and 13 Å. While immobilization of the plug domain with the shorter crosslinks resulted in a decrease in protein translocation, introduction of the longer crosslinkers allowed unrestricted protein translocation. These data suggest that the movement of

the plug domain needed for protein translocation is not more than 13 Å, which is substantially less than the 27 Å needed to reach the carboxyl-terminus of SecE. Additionally, molecular dynamics (MD) simulations were employed to examine molecular details on the position of the plug domain fixed with the longer crosslinkers. These simulations showed that the introduction of the 13 Å long crosslinker kept the plug domain at a stable position at the periplasmic side of TMS 7, clearing a vectorial path through the center of the protein translocation channel. A crystal structure of SecA-bound SecYEG representing a 'pre-open' state shows the plug domain has vacated its central position and is partly moved to the same location at TMS 7 as observed in our MD simulations. It is therefore plausible that this position is maintained during the translocation of secretory proteins.

The two helices of TMS2 and TMS 7 compose the lateral gate through which membrane spanning segments are thought to be inserted into the lipid bilayer. The location of the plug domain at the periplasmic side of the lateral gate might interfere with membrane protein insertion. In **Chapter 3** the plug domain was labeled site-specifically with the environment sensitive fluorophore NBD to monitor the conformational dynamics of the plug domain during the protein translocation and membrane protein insertion events. The NBD fluorophore exhibits a high fluorescence when exposed to a hydrophobic environment and the fluorescence is quenched in a hydrophilic environment. During protein translocation, we observed major fluorescence changes of NBD at different positions on the plug domain indicating a relocation from its original position to a more water-accessible environment. The relatively small changes suggest that the plug domain remains inside the channel, but moves away from the constriction ring. To obtain information on the plug domain dynamics when membrane proteins are inserted we used a ribosome with an exposed nascent chain of the FtsQ membrane segment that binds to SecYEG. The insertion of the membrane segment did not result in any fluorescence change of the NBD located at different positions of the plug domain, whereas control positions in the cytosolic loops recorded the efficient binding of the ribosome to SecYEG. This suggests that the plug domain stays at its original location during membrane protein insertion, at least during the initial stages of this process.

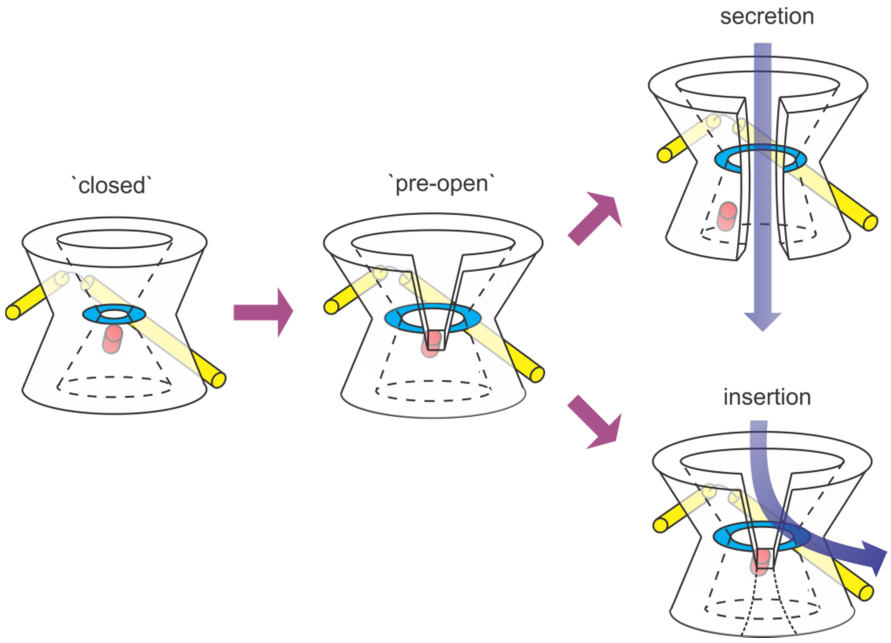

Figure 2: Model for the SecY channel structural rearrangements during membrane protein insertion and protein translocation. 1) The initial closed SecYEG translocon displays a constricted ring of hydrophobic residues (blue) with the plug domain (red) closely associated with it. 2) An intermediate 'pre-open' state showing an expansion of the funnel at the cytoplasmic side accompanied by a partial opening of the lateral gate. 3) The open state which occurs upon the binding of SecA. An incoming secretory polypeptide induces further opening of the channel and the plug domain vacates its original position to clear a vectorial path for the secretory preprotein. 4) An incoming hydrophobic polypeptide does not result in movement of the plug and the protein segment is guided into the lipid bilayer presumably along the surface of SecY possibly along a crevice at the lateral gate of SecY.

These data provide support for a model where during membrane protein insertion the plug domain closes a vectorial path through the channel and thereby guides nascent membrane proteins into the lipid bilayer (Figure 2). On the other hand, during protein translocation the plug domain vacates the position associated with the constriction ring and clears a path for the secretory protein to be translocated.

Flexible interaction between SecY and SecE

In **Chapter 4** we investigated the dynamics of the interaction of SecY and SecE during the protein translocation process. In the *M. jannaschii* SecYEβ crystal structure (Figure 1), the SecE subunit interacts at several positions with the two halves of the channel protein SecY, most notably at the tilted helix as well as with the amphipatic with SecY TMSs. During protein translocation the channel has to open substantially and it is plausible that SecE slides along the opening SecY channel thereby relocating interaction points. To explore this idea, we used a cysteine-based crosslinking approach to introduce disulfide bonds at SecY-E interaction points at the tilted helix and on the other side at the amphipatic helix. Analysis of protein translocation with the translocon restricted at a single contact point did not result in any inhibition. But when the translocation channel was immobilized at both sites of SecY-SecE interaction, a major reduction of the translocation activity occurred. This suggests that the interacting surface between SecE and SecY is flexible and that in case of channel opening, one or both of the contact surface slide along each other. Furthermore, we introduced protease cleavage sites in and around the hinge region of SecE to investigate the functional importance of this region. Cleavage of the hinge region significantly reduced protein translocation, whereas the cleavage directly N-terminal of the hinge region allowed full translocation activity. With the latter cleaved product, the SecY-SecE interaction is maintained at the two contact surfaces. This implies the hinge region is of essential importance for protein translocation, presumably by supporting the large conformational change of SecY during channel opening via flexible interactions.

Perspective

While most researchers focus their attention on the essential elements of the Sec machinery in order to understand the mechanism of protein translocation, it will also be important to obtain a complete picture. Recently, the crystal structure of the non-essential accessory SecDF complex has been solved, for the first time providing a glimpse of the possible mode of functioning of this

enigmatic protein complex. SecDF utilizes the proton motive force to facilitate a large conformational change of the periplasmic head domain, and it has been suggested that this large conformational change serves to pull preproteins out of the SecYEG channel at the trans side. How SecDF is associated with SecYEG is still poorly understood. Another accessory membrane protein, YidC interacts with SecYEG and SecDF and the question arises whether this multiprotein complex reflects a dynamic entity that undergoes complex dissociation depending on its specific function or whether it remains a stable entity throughout the catalytic cycle. How do these complexes respond to the binding of SecA or ribosomes? Both are SecYEG interacting partners and responsible for the different functions of the translocon, i.e., the secretion of proteins and the insertion of membrane proteins, respectively. Interplay between ribosome and SecA binding seems likely, but the mechanisms involved are unclear. For example inner membrane proteins with large carboxy-terminal periplasmic loops or domains depend on both the ribosome and SecA for assembly. How does this process occur? It is envisioned that the ribosome first docks to the SecYEG translocation channel inducing a conformational change (or a lack thereof regarding the plug domain) while continued translation promotes the insertion of the nascent chain into the lipid bilayer. At some point the ribosome complex needs to recognize that the emerging nascent chain contains a region that needs to be translocated. At that stage, the ribosome likely dissociates to allow SecA to bind to the translocating protein and to SecYEG. This in turn may initiate a different conformational change of SecYEG in order to promote translocation instead of insertion. To investigate these subtle transitional conformational changes most classical approaches lack the resolution needed to record these events. With the advance of the evolving technologies such processes can be defined and monitored with a greater precision both in in vitro systems as well as in living cells. For example, the advent of high resolution fluorescence microscopy is a promising tool for investigating protein translocation at a single molecule level. Further developments in single molecule measurements will result in a merge of scientific disciplines and with good statistical analysis eventually allow us understand the process of protein translocation at a detailed level. While collaborations between different branches of science are a challenge on itself, the potential outcome is promising.